

DYEING COMPOSITION CONTAINING A LACCASE AND METHOD FOR  
DYEING KERATINOUS FIBRES

The present invention relates to a composition for the oxidation dyeing of keratinous fibres comprising at least one enzyme of the laccase type, at least one oxidation dye and at least one particular alkalinizing agent as well as the methods of dyeing keratinous fibres, in particular human hair using this composition.

It is known to dye keratinous fibres, and in particular human hair, with dyeing compositions containing oxidation dye precursors, in particular ortho- and para-phenylenediamines, ortho- or para-aminophenols, heterocyclic bases generally called oxidation bases. The oxidation dye precursors, or oxidation bases, are colourless or weakly coloured compounds which, combined with oxidizing products, can give rise to coloured compounds by a process of oxidative condensation.

It is also known that the shades obtained with these oxidation bases can be varied by combining them with couplers or colour modifiers, the latter being chosen in particular from aromatic meta-diamines, meta-aminophenols, meta-diphenols and certain heterocyclic compounds.

The variety of molecules used in oxidation bases and couplers allows a rich palette of colours to be obtained.

The so-called "permanent" colour obtained by means of these oxidation dyes should moreover satisfy a number of requirements. Thus, it should have no drawbacks from the toxicological point of view, it 5 should make it possible to obtain shades of the desired intensity and it should exhibit good resistance towards external agents (light, adverse weather conditions, washing, permanent waving, perspiration, rubbing).

The dyes should also make it possible to 10 cover grey hair, and thus should be the least selective possible, that is to say they should make it possible to obtain the smallest possible differences in colour all along the same keratinous fibre, which may indeed be differently sensitized (i.e. damaged) between its 15 tip and its root.

The oxidation dyeing of keratinous fibres is generally carried out in an alkaline medium, in the presence of hydrogen peroxide. However, the use of alkaline media in the presence of hydrogen peroxide has 20 the disadvantage of causing substantial degradation of the fibres, as well as decolouring of the keratinous fibres which is not always desirable.

The oxidation dyeing of keratinous fibres can also be carried out with the aid of oxidizing systems 25 different from hydrogen peroxide such as enzymatic systems. Thus, it has already been proposed in Patent US 3,251,742, Patent Applications FR-A-2,112,549, FR-A-2,694,018, EP-A-0,504,005, WO95/07988, WO95/33836,

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WO95/33837, WO96/00290, WO97/19998 and WO97/19999 to dye keratinous fibres with compositions comprising at least one oxidation dye in combination with enzymes of the laccase type, the said compositions being brought 5 into contact with atmospheric oxygen. These dyeing formulations, although used under conditions which do not cause degradation of the keratinous fibres comparable to that caused by dyeings carried out in the presence of hydrogen peroxide, lead to colours which 10 are still inadequate both from the point of view of homogeneity of the colour distributed along the fibre ("unison"), from the point of view of chromaticity (luminosity) and of the dyeing power.

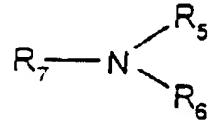
The aim of the present invention is to solve 15 the problems mentioned above.

The applicant has surprisingly discovered novel compositions containing, as oxidizing system, at least one enzyme of the laccase type and at least one particular alkalinizing agent which will be defined in 20 greater detail below, which may constitute, in the presence of oxidation dyes, ready-to-use dyeing formulations leading to colours which are more homogeneous, more intense and more chromatic without causing significant degradation or decolouring of the 25 keratinous fibres, exhibiting low selectivity and good resistance to various attacks to which the hair may be subjected.

These discoveries form the basis of the present invention.

The first subject of the present invention is therefore a ready-to-use composition intended for the 5 oxidation dyeing of keratinous fibres, in particular human keratinous fibres and more particularly human hair, comprising, in a carrier appropriate for dyeing keratinous fibres:

- (a) at least one enzyme of the laccase type;
- 10 - (b) at least one alkalinizing agent chosen from the group consisting of:
  - (i) a basic amino acid;
  - (ii) a compound of the following formula (A):  
 $X(OH)_n$  in which X represents K, Li when n=1; X  
 15 represents Mg, Ca when n=2; X represents  $N^+R_1R_2R_3R_4$  with  $R_1, R_2, R_3, R_4$ , which are identical or different, denoting a  $C_1-C_4$  alkyl radical, a  $C_1-C_4$  monohydroxyalkyl or  $C_2-C_4$  polyhydroxyalkyl radical, when n=1;
  - (iii) a compound of the following formula (B):



- 20 in which  $R_5$  denotes a  $C_1-C_6$  alkyl radical, a  $C_1-C_6$  monohydroxyalkyl or  $C_2-C_6$  polyhydroxyalkyl radical;  $R_6$ ,  $R_7$ , which are identical or different, denote a hydrogen atom, a  $C_1-C_6$  alkyl radical, a  $C_1-C_6$  monohydroxyalkyl or 25  $C_2-C_6$  polyhydroxyalkyl radical;
- with the proviso that

- R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub> do not simultaneously denote the C<sub>2</sub> β-hydroxy-alkyl radical,
  - if R<sub>6</sub> and R<sub>7</sub> simultaneously denote H, then R<sub>5</sub> does not denote a C<sub>2</sub> monohydroxyalkyl or branched C<sub>4</sub>
- 5 monohydroxyalkyl radical,
- if R<sub>5</sub> denotes hydrogen or a C<sub>1</sub>-C<sub>6</sub> alkyl radical and at the same time R<sub>6</sub> denotes a C<sub>1</sub>-C<sub>6</sub> alkyl radical, then R<sub>7</sub> does not denote H or a C<sub>1</sub>-C<sub>6</sub> alkyl radical;
- (iv) a compound of the following formula (C):
- R<sub>8</sub> \ N - W - N / R<sub>10</sub>  
                   |        |  
                  R<sub>9</sub>    R<sub>11</sub>      (C)
- 10 in which W is a propylene residue optionally substituted with a hydroxyl group or a C<sub>1</sub>-C<sub>4</sub> alkyl radical; R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub> and R<sub>11</sub>, which are identical or different, represent a hydrogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl or
- 15 C<sub>1</sub>-C<sub>4</sub> hydroxyalkyl radical;
- (c) at least one oxidation dye with the exception of autooxidizable indole dyes.

The laccase(s) used in the ready-to-use dye composition in accordance with the invention may be

20 chosen in particular from laccases of plant origin, animal origin, fungal origin (yeasts, moulds, fungi) or bacterial origin, organisms which may be of mono- or pluricellular origin. They can be obtained by biotechnology.

25 Among the laccases of plant origin which can be used according to the invention, there may be

mentioned the laccases produced by plants which perform chlorophyll synthesis as indicated in Application FR-A-2,694,018 such as those found in the extracts of Anacardiaceae such as for example the extracts of

- 5 *Magnifera indica*, *Schinus molle* or *Pleiogynium timoriense*, in the extracts of Podocarpaceae,  
Rosmarinus off., *Solanum tuberosum*, *Iris* sp., *Coffea* sp., *Daucus carota*, *Vinca minor*, *Persea americana*,  
*Catharenthus roseus*, *Musa* sp., *Malus pumila*, *Gingko*  
10 *biloba*, *Monotropa hypopithys* (Indian pipe), *Aesculus* sp., *Acer pseudoplatanus*, *Prunus persica*, *Pistacia*  
*palaestina*.

Among the laccases of fungal origin  
optionally obtained by biotechnology which can be used  
15 according to the invention, there may be mentioned the  
laccase(s) derived from *Polyporus versicolor*,  
*Rhizoctonia praticola* and *Rhus vernicifera* as  
indicated in Applications FR-A-2,112,549 and  
EP-A-504005, those described in Patent Application  
20 WO95/07988, WO95/33836, WO95/33837, WO96/00290,  
WO97/19998 and WO97/19999, whose content is an integral  
part of the present description, such as for example  
those derived from *Scytalidium*, *Polyporus pinsitus*,  
*Myceliophthora thermophila*, *Rhizoctonia solani*,  
25 *Pyricularia orizae*, or variants thereof. There may also  
be mentioned those derived from *Trametes versicolor*,  
*Fomes fomentarius*, *Chaetomium thermophile*, *Neurospora*  
*crassa*, *Coriolus versicol*, *Botrytis cinerea*,

Rigidoporus lignosus, Phellinus noxius, Pleurotus ostreatus, Aspergillus nidulans, Podospora anserina, Agaricus bisporus, Ganoderma lucidum, Glomerella cingulata, Lactarius piperatus, Russula delica,  
5 Heterobasidion annosum, Thelephora terrestris, Cladosporium cladosporioides, Cerrena unicolor, Coriolus hirsutus, Ceriporiopsis subvermispora, Coprinus cinereus, Panaeolus papilionaceus, Panaeolus sphinctrinus, Schizophyllum commune, Dichomitus squalens and variants thereof.

The laccases of fungal origin optionally obtained by biotechnology will be preferably chosen.

The enzymatic activity of the laccases of the invention which have syringaldazine among their substrates can be defined from the oxidation of syringaldazine under aerobic conditions. The lacu unit corresponds to the quantity of enzyme catalysing the conversion of 1 mmol of syringaldazine per minute at pH 5.5 at 30°C. The unit u corresponds to the quantity of enzyme producing a delta absorbance at 530 nm of 0.001 per minute using syringaldazine as substrate, at 30°C and at pH 6.5.  
15  
20

The enzymatic activity of the laccases of the invention can also be defined from the oxidation of para-phenylenediamine. The lacu unit corresponds to the quantity of enzyme producing a delta absorbance at 496.5 nm of 0.001 per minute using para-phenylenediamine as substrate (64 mM) at 30°C and at  
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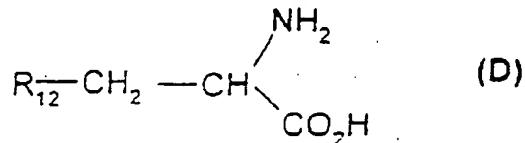
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pH 5. According to the invention, it is preferable to determine the enzymatic activity in lacu units.

The quantities of laccase used in the compositions of the invention will vary according to 5 the nature of the laccase chosen. Preferably, they will vary from 0.5 to 2000 lacu, or from 1000 to  $4 \times 10^7$  u units, or from 20 to  $2 \times 10^6$  <sup>ulac</sup> <sub>lacu</sub> units per 100 g of composition.

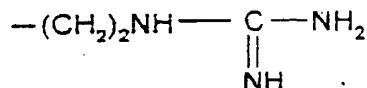
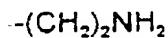
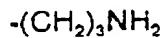
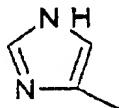
For the purposes of the present invention, 10 i.e. in the preceding text and in the text which follows, "basic amino acid" defines either (i) an amino acid having, in addition to the amine function positioned in  $\alpha$  with respect to the carboxyl group, an additional cationic (or basic) group; or (ii) an amino acid having a cationic (or basic) (hydrophilic) side chain; or (iii) an amino acid carrying a side chain 15 consisting of a nitrogenous base. These definitions are generally known and are published in general biochemistry books such as J.H. WEIL (1983) pages 5 and 20 the following pages, Lubert STRYER (1995) page 22, A. LEHNINGER (1993) pages 115-116, DE BOECK-WESMAEL (1994) pages 57-59.

The basic amino acids in accordance with the invention are preferably chosen from those 25 corresponding to the following formula (D):



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where R<sub>12</sub> denotes a group chosen from:



The compounds corresponding to formula (D) are histidine, lysine, ornithine, citrulline, arginine.

5 In the compounds of formula (A) or (B), according to the invention, the alkyl radicals may be linear or branched and the polyhydroxyalkyl radicals denote radicals comprising from 2 to 6 hydroxyl groups and preferably from 2 to 4.

10 The compounds of formula (B), according to the invention, are preferably chosen from the group consisting of diethanolamine, monoisopropanolamine, diisopropanolamine, triisopropanolamine, 2-amino-2-methyl-1,3-propanediol, 2-amino-2-ethyl-1,3-propane-15 diol, 2-amino-1-n-butanol, 1-diethylamino-2,3-propane-diol, tris(hydroxymethyl)aminomethane, ethylmonoethanolamine.

The compositions in accordance with the invention contain the particular alkalinizing agents defined above in contents by weight which may range from 0.001% to 20%, preferably from 0.01% to 5% and

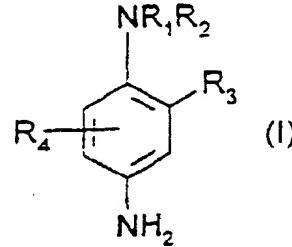
- 5 still more preferably from 0.05% to 3%, relative to the total weight of the composition.

The nature of the oxidation dye(s) used in the ready-to-use dyeing composition is not critical. They are chosen from oxidation bases and/or couplers.

- 10 The oxidation bases may be chosen in particular from para-phenylenediamines, double bases, para-aminophenols, ortho-aminophenols and heterocyclic oxidation bases.

- 15 Among the para-phenylenediamines which can be used as oxidation base in the dyeing composition in accordance with the invention, there may be mentioned in particular the compounds of the following formula

(I) and their addition salts with an acid:



- 20 in which:

- R<sub>1</sub> represents a hydrogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl radical, a monohydroxy(C<sub>1</sub>-C<sub>4</sub> alkyl) radical, a polyhydroxy-(C<sub>2</sub>-C<sub>4</sub> alkyl) radical, a (C<sub>1</sub>-C<sub>4</sub>)alkoxy(C<sub>1</sub>-C<sub>4</sub>)alkyl radical, a C<sub>1</sub>-C<sub>4</sub> alkyl radical substituted with a

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nitrogen-containing group, a phenyl radical or a  
4'-aminophenyl radical;

- R<sub>2</sub> represents a hydrogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl radical, a monohydroxy(C<sub>1</sub>-C<sub>4</sub> alkyl) radical, a polyhydroxy(C<sub>2</sub>-C<sub>4</sub> alkyl) radical, a (C<sub>1</sub>-C<sub>4</sub>)alkoxy(C<sub>1</sub>-C<sub>4</sub>)alkyl radical or a C<sub>1</sub>-C<sub>4</sub> alkyl radical substituted with a nitrogen-containing group;
  - R<sub>3</sub> represents a hydrogen atom, a halogen atom such as a chlorine, bromine, iodine or fluorine atom, a C<sub>1</sub>-C<sub>4</sub> alkyl radical, a monohydroxy(C<sub>1</sub>-C<sub>4</sub> alkyl) radical, a hydroxy(C<sub>1</sub>-C<sub>4</sub> alkoxy) radical, an acetylamino(C<sub>1</sub>-C<sub>4</sub> alkoxy) radical, a mesylamino(C<sub>1</sub>-C<sub>4</sub> alkoxy) radical or a carbamoylamino(C<sub>1</sub>-C<sub>4</sub> alkoxy) radical,
  - R<sub>4</sub> represents a hydrogen or halogen atom or a C<sub>1</sub>-C<sub>4</sub> alkyl radical.

Among the nitrogen-containing groups of formula (I) above, there may be mentioned in particular the amino, mono( $C_1-C_4$ )alkylamino, ( $C_1-C_4$ )dialkylamino, ( $C_1-C_4$ )trialkylamino, monohydroxy( $C_1-C_4$ )alkylamino, 20 imidazolinium and ammonium radicals.

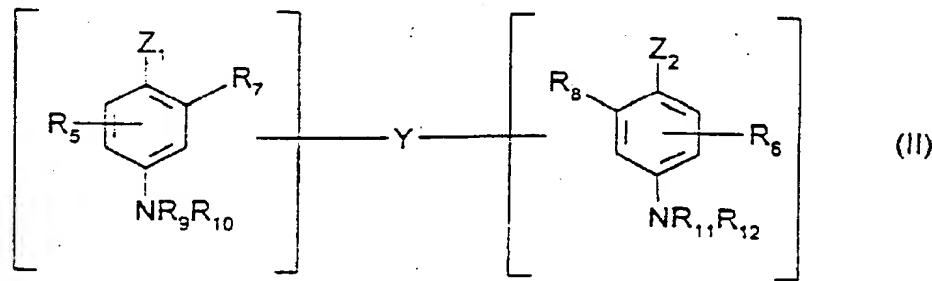
Among the para-phenylenediamines of formula (I) above, there may be mentioned more particularly para-phenylenediamine, para-tolylenediamine, 2-chloro-para-phenylenediamine, 2,3-dimethyl-para-phenylene-25 diamine, 2,6-dimethyl-para-phenylenediamine, 2,6-diethyl-para-phenylenediamine, 2,5-dimethyl-para-phenylenediamine, N,N-dimethyl-para-phenylenediamine, N,N-diethyl-para-phenylenediamine, N,N-dipropyl-para-

- phenylenediamine, 4-amino-N,N-diethyl-3-methylaniline,  
N,N-bis( $\beta$ -hydroxyethyl)-para-phenylenediamine, 4-N,N-  
bis( $\beta$ -hydroxyethyl)amino-2-methylaniline, 4-N,N-bis( $\beta$ -  
hydroxyethyl)amino-2-chloroaniline, 2- $\beta$ -hydroxyethyl-  
5 para-phenylenediamine, 2-fluoro-para-phenylenediamine,  
2-isopropyl-para-phenylenediamine, N-( $\beta$ -hydroxypropyl)-  
para-phenylenediamine, 2-hydroxymethyl-para-phenylene-  
diamine, N,N-dimethyl-3-methyl-para-phenylenediamine,  
N,N-(ethyl- $\beta$ -hydroxyethyl)-para-phenylenediamine,  
10 N-( $\beta$ , $\gamma$ -dihydroxypropyl)-para-phenylenediamine, N-(4'-  
aminophenyl)-para-phenylenediamine, N-phenyl-para-  
phenylenediamine, 2- $\beta$ -hydroxyethoxy-para-  
phenylenediamine, 2- $\beta$ -acetylamoethoxy-para-  
phenylenediamine, N-( $\beta$ -methoxyethyl)-para-  
15 phenylenediamine, and their addition salts with an  
acid.

Among the para-phenylenediamines of formula  
(I) above, there are most particularly preferred para-  
phenylenediamine, para-tolylenediamine, 2-isopropyl-  
20 para-phenylenediamine, 2- $\beta$ -hydroxyethyl-para-  
phenylenediamine, 2- $\beta$ -hydroxyethoxy-para-phenylene-  
diamine, 2,6-dimethyl-para-phenylenediamine,  
2,6-diethyl-para-phenylenediamine, 2,3-dimethyl-para-  
phenylenediamine, N,N-bis( $\beta$ -hydroxyethyl)-para-  
25 phenylenediamine, 2-chloro-para-phenylenediamine, 2- $\beta$ -  
acetylamoethoxy-para-phenylenediamine, and their  
addition salts with an acid.

According to the invention, "double bases" is understood to mean the compounds containing at least two aromatic rings on which amino and/or hydroxyl groups are carried.

5 Among the double bases which can be used as oxidation bases in the dyeing compositions in accordance with the invention, there may be mentioned in particular the compounds corresponding to the following formula (II), and their addition salts with  
10 an acid:



in which:

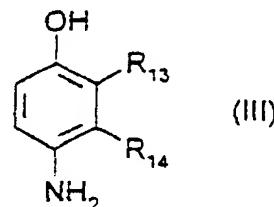
- $Z_1$  and  $Z_2$ , which are identical or different, represent a hydroxyl or  $-NH_2$  radical which may be  
15 substituted with a  $C_1-C_4$  alkyl radical or with a linking arm  $Y$ ;
- the linking arm  $Y$  represents a linear or branched alkylene chain comprising from 1 to 14 carbon atoms, which may be interrupted by or which may end with one  
20 or more nitrogen-containing groups and/or one or more heteroatoms such as oxygen, sulphur or nitrogen atoms, and optionally substituted with one or more hydroxyl or  $C_1-C_6$  alkoxy radicals;

- R<sub>5</sub> and R<sub>6</sub> represent a hydrogen or halogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl radical, a monohydroxy(C<sub>1</sub>-C<sub>4</sub> alkyl) radical, a polyhydroxy(C<sub>2</sub>-C<sub>4</sub> alkyl) radical, an amino(C<sub>1</sub>-C<sub>4</sub> alkyl) radical or a linking arm Y;
- 5 - R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub> and R<sub>12</sub>, which are identical or different, represent a hydrogen atom, a linking arm Y or a C<sub>1</sub>-C<sub>4</sub> alkyl radical;  
it being understood that the compounds of formula (II) contain only one linking arm Y per molecule.
- 10 Among the nitrogen-containing groups of formula (II) above, there may be mentioned in particular the amino, mono(C<sub>1</sub>-C<sub>4</sub>)alkylamino, (C<sub>1</sub>-C<sub>4</sub>)dialkylamino, (C<sub>1</sub>-C<sub>4</sub>)trialkylamino, monohydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylamino, imidazolinium and ammonium radicals.
- 15 Among the double bases of formulae (II) above, there may be mentioned more particularly N,N'-bis(β-hydroxyethyl)-N,N'-bis(4'-aminophenyl)-1,3-diaminopropanol, N,N'-bis(β-hydroxyethyl)-N,N'-bis(4'-aminophenyl)ethylenediamine, N,N'-bis(4-aminophenyl)-tetramethylenediamine, N,N'-bis(β-hydroxyethyl)-N,N'-bis(4-aminophenyl)tetramethylenediamine, N,N'-bis(4-methylaminophenyl)tetramethylenediamine, N,N'-bis(ethyl)-N,N'-bis(4'-amino-3'-methylphenyl)ethylene-diamine, 1,8-bis(2,5-diaminophenoxy)-3,5-dioxaoctane, and their addition salts with an acid.

Among these double bases of formula (II), N,N'-bis(β-hydroxyethyl)-N,N'-bis(4'-aminophenyl)-1,3-

diaminopropanol, 1,8-bis(2,5-diaminophenoxy)-3,5-dioxaoctane or one of their addition salts with an acid are particularly preferred.

Among the para-aminophenols which can be used 5 as oxidation bases in the dyeing compositions in accordance with the invention, there may be mentioned in particular the compounds corresponding to the following formula (III), and their addition salts with an acid:



10

in which:

- R<sub>13</sub> represents a hydrogen or halogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl, monohydroxy(C<sub>1</sub>-C<sub>4</sub> alkyl), (C<sub>1</sub>-C<sub>4</sub>)alkoxy(C<sub>1</sub>-C<sub>4</sub>)-alkyl, amino(C<sub>1</sub>-C<sub>4</sub> alkyl) or hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylamino-15 (C<sub>1</sub>-C<sub>4</sub> alkyl) radical,
  - R<sub>14</sub> represents a hydrogen or halogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl, monohydroxy(C<sub>1</sub>-C<sub>4</sub> alkyl), polyhydroxy(C<sub>2</sub>-C<sub>4</sub> alkyl), amino(C<sub>1</sub>-C<sub>4</sub> alkyl), cyano(C<sub>1</sub>-C<sub>4</sub> alkyl) or (C<sub>1</sub>-C<sub>4</sub>)alkoxy(C<sub>1</sub>-C<sub>4</sub>)alkyl radical,
- 20 it being understood that at least one of the radicals R<sub>13</sub> or R<sub>14</sub> represents a hydrogen atom.

Among the para-aminophenols of formula (III) above, there may be mentioned more particularly para-aminophenol, 4-amino-3-methylphenol, 4-amino-3-fluorophenol, 4-amino-3-hydroxymethylphenol, 4-amino-2-

methylphenol, 4-amino-2-hydroxymethylphenol, 4-amino-2-methoxymethylphenol, 4-amino-2-aminomethylphenol, 4-amino-2-( $\beta$ -hydroxyethylaminomethyl)phenol, 4-amino-2-fluorophenol, and their addition salts with an acid.

5 Among the ortho-aminophenols which can be used as oxidation bases in the dyeing compositions in accordance with the invention, there may be mentioned more particularly 2-aminophenol, 2-amino-5-methylphenol, 2-amino-6-methylphenol, 5-acetamido-2-aminophenol, and their addition salts with an acid.

10 Among the heterocyclic bases which can be used as oxidation bases in the dyeing compositions in accordance with the invention, there may be mentioned more particularly pyridine derivatives, pyrimidine derivatives, pyrazole derivatives, pyrazolopyrimidine derivatives, and their addition salts with an acid.

15 Among the pyridine derivatives, there may be mentioned more particularly the compounds described for example in Patents GB 1,026,978 and GB 1,153,196, such as 2,5-diaminopyridine, 2-(4-methoxyphenyl)amino-3-aminopyridine, 2,3-diamino-6-methoxypyridine, 2-( $\beta$ -methoxyethyl)amino-3-amino-6-methoxypyridine, 3,4-diaminopyridine, and their addition salts with an acid.

20 25 Among the pyrimidine derivatives, there may be mentioned more particularly the compounds described for example in German Patent DE 2,359,399 or Japanese Patents JP 88-169,571 and JP 91-333,495 or Patent

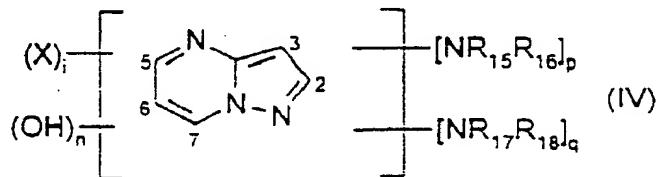
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Application WO 96/15765, such as 2,4,5,6-tetra-aminopyrimidine, 4-hydroxy-2,5,6-triaminopyrimidine, 2-hydroxy-4,5,6-triaminopyrimidine, 2,4-dihydroxy-5,6-diaminopyrimidine, 2,5,6-triaminopyrimidine, and their  
5 addition salts with an acid.

Among the pyrazole derivatives, there may be mentioned more particularly the compounds described in Patents DE 3,843,892, DE 4,133,957 and Patent Applications WO 94/08969, WO 94/08970, FR-A-2,733,749 and DE 195 43 988 such as 4,5-diamino-1-methylpyrazole, 3,4-diaminopyrazole, 4,5-diamino-1-(4'-chlorobenzyl)-pyrazole, 4,5-diamino-1,3-dimethylpyrazole, 4,5-diamino-3-methyl-1-phenylpyrazole, 4,5-diamino-1-methyl-3-phenylpyrazole, 4-amino-1,3-dimethyl-5-  
15 hydrazinopyrazole, 1-benzyl-4,5-diamino-3-methyl-pyrazole, 4,5-diamino-3-tert-butyl-1-methylpyrazole, 4,5-diamino-1-tert-butyl-3-methylpyrazole, 4,5-diamino-1-( $\beta$ -hydroxyethyl)-3-methylpyrazole, 4,5-diamino-1-ethyl-3-methylpyrazole, 4,5-diamino-1-ethyl-3-(4'-  
20 methoxyphenyl)pyrazole, 4,5-diamino-1-ethyl-3-hydroxymethylpyrazole, 4,5-diamino-3-hydroxymethyl-1-methylpyrazole, 4,5-diamino-3-hydroxymethyl-1-isopropylpyrazole, 4,5-diamino-3-methyl-1-isopropylpyrazole, 4-amino-5-(2'-aminoethyl)amino-1,3-dimethyl-  
25 pyrazole, 3,4,5-triaminopyrazole, 1-methyl-3,4,5-triaminopyrazole, 3,5-diamino-1-methyl-4-methylamino-pyrazole, 3,5-diamino-4-( $\beta$ -hydroxyethyl)amino-1-methylpyrazole, and their addition salts with an acid.

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Among the pyrazolopyrimidine derivatives,  
 there may be mentioned more particularly the  
 pyrazolo[1,5-a]pyrimidines of the following formula  
 (IV), their addition salts with an acid or with a base  
 5 and their tautomeric forms, when a tautomeric  
 equilibrium exists:

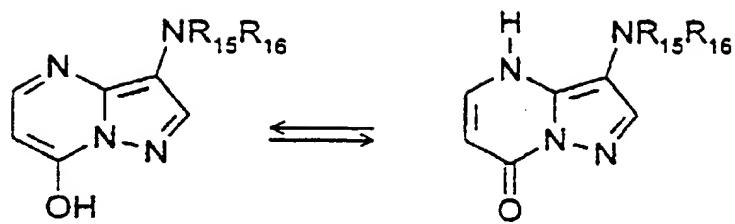


in which:

- $R_{15}$ ,  $R_{16}$ ,  $R_{17}$  and  $R_{18}$ , which are identical or  
 10 different, denote a hydrogen atom, a  $C_1-C_4$  alkyl  
 radical, an aryl radical, a  $C_1-C_4$  hydroxyalkyl  
 radical, a  $C_2-C_4$  polyhydroxyalkyl radical, a  
 15  $(C_1-C_4)$  alkoxy( $C_1-C_4$  alkyl) radical, a  $C_1-C_4$  aminoalkyl  
 radical (it being possible for the amine to be  
 protected with an acetyl, ureido or sulphonyl  
 radical), a  $(C_1-C_4)$  alkylamino( $C_1-C_4$  alkyl) radical, a  
 20 di-[ $(C_1-C_4)$  alkyl]amino( $C_1-C_4$  alkyl) radical (it being  
 possible for the dialkyl radicals to form a carbon-  
 containing ring or a 5- or 6-membered heterocycle),  
 a hydroxy( $C_1-C_4$ )alkyl- or di-[hydroxy( $C_1-C_4$ )alkyl]-  
 25 amino( $C_1-C_4$  alkyl) radical,
- the  $X$  radicals, which are identical or different,  
 denote a hydrogen atom, a  $C_1-C_4$  alkyl radical, an  
 aryl radical, a  $C_1-C_4$  hydroxyalkyl radical, a  $C_2-C_4$   
 polyhydroxyalkyl radical, a  $C_1-C_4$  aminoalkyl radical,

- a ( $C_1-C_4$ )alkylamino( $C_1-C_4$  alkyl) radical, a di-[ $(C_1-C_4)$ alkyl]amino( $C_1-C_4$  alkyl) radical (it being possible for the dialkyls to form a carbon-containing ring or a 5- or 6-membered heterocycle),
- 5 a hydroxy( $C_1-C_4$ )alkyl or di-[hydroxy( $C_1-C_4$ )alkyl]-amino( $C_1-C_4$  alkyl) radical, an amino radical, a ( $C_1-C_4$ )alkyl- or di-[ $(C_1-C_4)$ alkyl]-amino radical; a halogen atom, a carboxylic acid group, a sulphonic acid group;
- 10 - i equals 0, 1, 2 or 3;
- p equals 0 or 1;
  - q equals 0 or 1;
  - n equals 0 or 1;
- with the proviso that:
- 15 - the sum p + q is different from 0;
- when p + q is equal to 2, then n equals 0 and the groups  $NR_{15}R_{16}$  and  $NR_{17}R_{18}$  occupy positions (2,3); (5,6); (6,7); (3,5) or (3,7);
  - when p + q is equal to 1, then n equals 1 and the
- 20 group  $NR_{15}R_{16}$  (or  $NR_{17}R_{18}$ ) and the OH group occupy positions (2,3); (5,6); (6,7); (3,5) or (3,7).

When the pyrazolo[1,5-a]pyrimidines of formula (IV) above are such that they comprise a hydroxyl group on one of the positions 2, 5 or 7 at the 25  $\alpha$  position with respect to a nitrogen atom, a tautomeric equilibrium exists which is represented for example by the following scheme:



Among the pyrazolo[1,5-a]pyrimidines of formula (IV) above, there may be mentioned in particular:

- 5 - pyrazolo[1,5-a]pyrimidine-3,7-diamine;
- 2,5-dimethylpyrazolo[1,5-a]pyrimidine-3,7-diamine;
- pyrazolo[1,5-a]pyrimidine-3,5-diamine;
- 2,7-dimethylpyrazolo[1,5-a]pyrimidine-3,5-diamine;
- 3-aminopyrazolo[1,5-a]pyrimidin-7-ol;
- 10 - 3-aminopyrazolo[1,5-a]pyrimidin-5-ol;
- 2-(3-aminopyrazolo[1,5-a]pyrimidin-7-ylamino)ethanol;
- 2-(7-aminopyrazolo[1,5-a]pyrimidin-3-ylamino)ethanol;
- 2-[(3-aminopyrazolo[1,5-a]pyrimidin-7-yl)(2-hydroxyethyl)amino]ethanol;
- 15 - 2-[(7-aminopyrazolo[1,5-a]pyrimidin-3-yl)(2-hydroxyethyl)amino]ethanol;
- 5,6-dimethylpyrazolo[1,5-a]pyrimidine-3,7-diamine;
- 2,6-dimethylpyrazolo[1,5-a]pyrimidine-3,7-diamine;
- 2,5,N7,N7-tetramethylpyrazolo[1,5-a]pyrimidine-3,7-
- 20 diamine;

and their addition salts and their tautomeric forms, when a tautomeric equilibrium exists.

The pyrazolo[1,5-a]pyrimidines of formula (IV) above may be prepared by cyclization from an

aminopyrazole according to the syntheses described in the following references:

- EP 628559 BEIERSDORF-LILLY
- R. Vishdu, H. Navedul, Indian J. Chem., 34b(6), 514,  
5 1995.
- N.S. Ibrahim, K.U. Sadek, F.A. Abdel-Al, Arch.  
Pharm., 320, 240, 1987.
- R.H. Springer, M.B. Scholten, D.E. O'Brien,  
T. Novinson, J.P. Miller, R.K. Robins, J. Med.  
10 Chem., 25, 235, 1982.
- T. Novinson, R.K. Robins, T.R. Matthews, J. Med.  
Chem., 20, 296, 1977.
- US 3907799 ICN PHARMACEUTICALS

The pyrazolo[1,5-a]pyrimidines of formula  
15 (IV) above can also be prepared by cyclization from  
hydrazine according to the syntheses described in the  
following references:

- A. McKillop and R.J. Kobilecki, Heterocycles, 6(9),  
1355, 1977.
- E. Alcade, J. De Mendoza, J.M. Marcia-Marquina,  
C. Almera, J. Elguero, J. Heterocyclic Chem., 11(3),  
20 423, 1974.
- K. Saito, I. Hori, M. Higarashi, H. Midorikawa,  
Bull. Chem. Soc. Japan, 47(2), 476, 1974.

25 The oxidation base(s) preferably represent  
from 0.0005 to 12% by weight approximately of the total  
weight of the dyeing composition in accordance with the

invention, and still more preferably from 0.005 to 6% by weight approximately of this weight.

The coupler(s) which can be used in the ready-to-use dyeing composition in accordance with the invention are those conventionally used in oxidation dyeing compositions, that is to say meta-phenylene-diamines, meta-aminophenols, meta-diphenols, heterocyclic couplers, and their addition salts with an acid.

These couplers may be chosen in particular from 2-methyl-5-aminophenol, 5-N-( $\beta$ -hydroxyethyl)amino-2-methylphenol, 3-aminophenol, 1,3-dihydroxybenzene, 1,3-dihydroxy-2-methylbenzene, 4-chloro-1,3-dihydroxybenzene, 2,4-diamino-1-( $\beta$ -hydroxyethoxy)benzene, 2-amino-4-( $\beta$ -hydroxyethylamino)-1-methoxybenzene, 1,3-diaminobenzene, 1,3-bis(2,4-diaminophenoxy)propane, sesamol,  $\alpha$ -naphthol, 6-hydroxyindole, 4-hydroxyindole, 4-hydroxy-N-methylindole, 6-hydroxyindoline, 2,6-dihydroxy-4-methylpyridine, 1-H-3-methylpyrazol-5-one, 1-phenyl-3-methylpyrazol-5-one, 2,6-dimethylpyrazolo[1,5-b]-1,2,4-triazole, 2,6-dimethyl[3,2-c]-1,2,4-triazole, 6-methylpyrazolo[1,5-a]benzimidazole, and their addition salts with an acid.

These couplers preferably represent from 0.0001 to 10% by weight approximately of the total weight of the ready-to-use dyeing composition, and still more preferably from 0.005 to 5% by weight approximately of this weight.

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In general, the addition salts with an acid which can be used in the context of the dyeing compositions of the invention (oxidation bases and couplers) are in particular chosen from hydrochlorides, 5 hydrobromides, sulphates and tartrates, lactates and acetates.

The dyeing composition of the invention may also contain, in addition to the oxidation dyes defined above, direct dyes in order to increase the shimmer of 10 the shades. These direct dyes can then in particular be chosen from nitro, azo or anthraquinone dyes.

The ready-to-use dyeing composition in accordance with the invention may also contain various adjuvants conventionally used in hair dyeing 15 compositions, such as anionic, cationic, nonionic, amphoteric or zwitterionic surfactants or mixtures thereof, polymers, thickeners, antioxidants, enzymes different from the laccases used in accordance with the invention, such as for example peroxidases or oxido- 20 reductases containing 2 electrons, penetrating agents, sequestering agents, perfumes, dispersing agents, film-forming agents, screening agents, vitamins, preservatives or opacifying agents.

Of course, persons skilled in the art will be 25 careful to choose this or these optional additional compounds such that the advantageous properties intrinsically attached to the ready-to-use dyeing composition in accordance with the invention are not,

or substantially not, impaired by the addition(s) envisaged.

The ready-to-use dyeing composition in accordance with the invention can be provided in 5 various forms, such as in the form of liquids, creams, gels, optionally pressurized, or in any other form appropriate for dyeing keratinous fibres, in particular human hair. In this case, the oxidation dye(s) and the laccase(s) are present in the same ready-to-use 10 composition, and consequently the said composition should be free of gaseous oxygen, so as to avoid any premature oxidation of the oxidation dye(s).

The subject of the invention is also a method of dyeing keratinous fibres, and in particular human 15 keratinous fibres such as hair, using the ready-to-use dyeing composition as defined above.

According to this method, at least one ready-to-use dyeing composition as defined above is applied to the fibres for a sufficient time to develop the 20 desired colour, after which they are rinsed, optionally washed with shampoo, rinsed again and dried.

The time necessary for the development of the colour on the keratinous fibres is generally between 3 and 60 minutes and still more precisely 5 and 25 40 minutes.

According to one particular embodiment of the invention, the method comprises a preliminary step consisting in storing in a separate form, on the one

hand, a composition (A) comprising, in a medium appropriate for dyeing, at least one oxidation dye as defined above and, on the other hand, a composition (B) containing, in a medium appropriate for dyeing, at 5 least one enzyme of the laccase type and at least one particular alkalinizing agent as defined above, and then in mixing them at the time of use before applying this mixture to the keratinous fibres.

According to a specific embodiment of the 10 invention, the alkalinizing agent may be incorporated into the composition (A).

Another subject of the invention is a multi-compartment device or dyeing (kit) or any other multi-compartment packaging system in which a first 15 compartment contains the composition (A) as defined above and a second compartment contains a composition (B) as defined above. These devices may be equipped with a means which makes it possible to deliver the desired mixture to the hair, such as the devices 20 described in Patent FR-2,586,913 in the name of the applicant.

The medium appropriate for keratinous fibres (or carrier) of the dyeing compositions in accordance with the invention generally consists of water or of a 25 mixture of water and of at least one organic solvent in order to solubilize the compounds which might not be sufficiently soluble in water. As organic solvent, there may be mentioned for example C<sub>1</sub>-C<sub>4</sub> alkanols such

as ethanol and isopropanol as well as aromatic alcohols such as benzyl alcohol, similar products and mixtures thereof.

The solvents may be present in proportions  
5 preferably of between 1 and 40% by weight approximately relative to the total weight of the dyeing composition, and still more preferably between 5 and 30% by weight approximately.

The pH of the dyeing compositions in  
10 accordance with the invention is chosen such that the enzymatic activity of the laccase is not impaired. It varies generally from 6 to 11 approximately, and more preferably from 6 to 9 approximately.

Concrete examples illustrating the invention  
15 will now be given.

In the text which follows and in the preceding text, unless otherwise stated, the percentages are expressed by weight.

The examples below illustrate the invention  
20 with no limitation being implied.

**EXAMPLE 1**                   **Dyeing composition**

The following ready-to-use dyeing composition was prepared (contents in grams):

- Laccase obtained from *Rhus vernicifera*                   1.8 g containing 180 units/mg units/mg, marketed by the company SIGMA
- ( $C_8-C_{10}$ )Alkyl polyglucoside in aqueous solution containing 60% of active                   8.0 g

substance (AS) sold under the name ORAMIX

CG110 by the company SEPPIC

- Paraphenylenediamine	0.254 g
- 2,4-Diaminophenoxyethanol dihydrochloride	0.260 g
- Arginine	qs pH 6.5
- Demineralized water	qs 100 g

This ready-to-use dyeing composition was applied to locks of natural grey hair which is 90% white for 40 minutes at 30°C. The hair was then rinsed, washed with a standard shampoo and then dried.

5 Locks of hair with bluish grey colour were obtained.

In this example, 1.8 g of laccase obtained from *Rhus vernicifera* containing 180 units/mg can be replaced by 1 g of laccase obtained from *Pyricularia* 10 *Orizae* containing 100 units/mg sold by the company I.C.N.

#### EXAMPLE 2: Dyeing composition

The following ready-to-use dyeing composition was prepared (contents in grams):

- Laccase obtained from <i>Rhus vernicifera</i> containing 180 units/mg units/mg, marketed by the company SIGMA	1.8 g
- ( $C_8-C_{10}$ ) Alkyl polyglucoside in aqueous solution containing 60% of active substance (AS) sold under the name ORAMIX CG110 by the company SEPPIC	8.0 g
- Paraphenylenediamine	0.254 g

- 2,4-Diaminophenoxyethanol dihydrochloride	0.260 g
- Ethanol	20.0 g
- Citrulline	qs pH 8.0
- Demineralized water	qs 100 g

This ready-to-use dyeing composition was applied to locks of natural grey hair which is 90% white for 40 minutes at 30°C. The hair was then rinsed, washed with a standard shampoo and then dried.

- 5 Locks of hair with bluish grey colour were obtained.

In this example, 1.8 g of laccase obtained from *Rhus vernicifera* containing 180 units/mg can be replaced by 1 g of laccase obtained from *Pyricularia Orizae* containing 100 units/mg sold by the company I.C.N.